

**THERAPEUTIC AND DIAGNOSTIC COMPOUNDS,
COMPOSITIONS, AND METHODS**

Related Applications

5 This application is a continuation under 35 U.S.C. 111(a) of International Application Serial No. PCT/US02/00629 filed 08 January 2002 and published in English as WO 02/062398 on 15 August 2002, which claims priority from U.S. Provisional Application Serial No. 60/260,349 filed 07 January 2001, which applications and publication are incorporated herein by reference.

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Background of the Invention

 The invasive growth of cancer cells into bone tissues often results in severe pain syndromes and may ultimately result in death. Ionizing radiation has been used to achieve a reduction in pain. In particular, external radiation
15 therapy has been applied in cases where bone pain is restricted to focal areas. For disseminated bone metastases, however, a treatment which focuses the radiation dose to bone tissue and particularly to bone metastases is desirable.

 Prior to therapy it is necessary to obtain reliable diagnostic information and to this end several approaches have been tried. An approach
20 that has been the subject of a number of patents, for example, U.S. Pat. No. 3,974,268 to Subramanian et al., involves the use of a technetium-99m diphosphonate complex as a skeletal imaging agent. This approach utilizes a diphosphonate as both the bone-seeking agent and the complexing agent for the radionuclide. Such an approach has at least three major disadvantages. First, by
25 involving the bone-seeking agent in the complexation of the metal radionuclide, the ability of the agent to direct, or "target," the radionuclide to the desired site may be diminished. Second, because diphosphonate salts or chelates are known to form polymers, a preparation of diphosphonates and radionuclides typically includes multiple forms of diphosphonate-radionuclide complexes which have
30 differing charge and uptake properties. Third, the complex formed between a

diphosphonate and a radionuclide is not of optimal stability which may necessitate purification of the diphosphonate complex prior to use. Further, even a purified diphosphonate complex may lose the radionuclide during its use.

U.S. Patent No. 4,853,209, is directed to the use of Sm-153,
5 Gd-159, or Ho-166 complexed with a ligand selected from
ethylenediaminetetramethylene-phosphonic acid (EDTMP), diethylenetriamine-
pentamethylenephosphonic acid (DTPMP), hydroxyethylethylenediamine-
trimethylenephosphonic acid (HEEDTMP), nitrilotrimethylenephosphonic acid
(NTMP), and tris(2-aminoethyl)aminehexamethylenephosphonic acid (TTHMP),
10 to suppress bone marrow growth.

U.S. Patent No. 4,882,142, is directed to a method for the
suppression of bone marrow and to a composition for use in the method. The
method comprises administering a bone marrow suppressing amount of at least
one composition comprised of a radionuclide Sm-153, Gd-159, or Ho-166,
15 complexed with 1,4,7,10-tetraazacyclododecanemethylenephosphonic acid as the
macrocyclic chelating moiety.

U.S. Patent No. 5,059,412 is directed to compositions comprising
a particle emitting radionuclide complexed with a macrocyclic aminophosphonic
acid, wherein the nitrogen and phosphorous are interconnected by an alkylene
20 group.

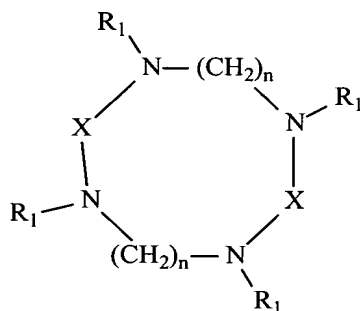
U.S. Patent Nos. 5,202,109 and 5,089,249 are directed to
conjugates formed from calcified tissue targeting agents and radiolabeled
compounds. One conjugate comprises a chelate and a targeting agent that is
capable of associating with calcified tissue, wherein the chelate contains at least
25 one nitrogen atom or at least one sulfur atom, or a combination thereof.

In spite of the above disclosures, a continuing need exists for
therapeutic and diagnostic agents that are useful for bone marrow suppression,
cancer therapy, treating bone pain, and as diagnostic agents. Preferred agents
may possess improved stability, improved uptake in bone, or improved retention
30 in bone.

Summary of the Invention

Applicant has discovered a group of structurally distinct radionuclide complexes that target bone, and thus, are useful as diagnostic (e.g., imaging) agents and as therapeutic agents (e.g., for bone marrow suppression, cancer therapy, treating bone pain, or treating other bone related diseases).
 Accordingly, the invention provides a complex of the invention comprising:

a) compound of formula I:

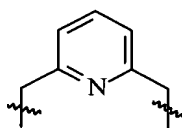


(I)

wherein:

each R_1 is independently hydrogen or (C_1-C_4) alkyl, optionally substituted with carboxy (COOH);

each X is independently $(CH_2)_n$ or



and each n is independently 2, 3, or 4;

wherein the compound of formula I is substituted on one or more (e.g. 1, 2, 3, or 4) carbons other than a carbon of R_1 with one or more (e.g., 1, 2, 3, or 4) groups $-Y(PO_3H_2)_m$; wherein Y is a linker group; and m is 1, 2, 3, 4, 5, or 6; or a pharmaceutically acceptable salt thereof; and

b) a detectable or therapeutic radionuclide.

The invention also provides a method for detecting the presence or absence of a calcified tissue target site within a mammal, comprising: administering to the mammal a detectable dose of a complex of the invention; and detecting the compound in the mammal to determine the presence or absence
5 of the target site.

The invention also provides a therapeutic method for treating a bone related disease in a mammal in need of such therapy comprising administering to the mammal, an effective amount of a complex of the invention comprising a therapeutic radionuclide.

10 The invention also provides a therapeutic method for suppressing bone marrow in a mammal in need of such therapy comprising administering to the mammal, an effective bone marrow suppressing amount of a complex of the invention comprising a therapeutic radionuclide.

The invention also provides a therapeutic method for treating
15 cancer in a mammal in need of such therapy comprising administering to the mammal, an effective amount of a complex of the invention comprising a therapeutic radionuclide.

The invention also provides a therapeutic method for treating bone pain in a mammal in need of such therapy comprising administering to the
20 mammal, an effective amount of a complex of the invention comprising a therapeutic radionuclide.

The invention also provides a pharmaceutical composition comprising a complex of the invention and a pharmaceutically acceptable carrier.

The invention also provides a complex of the invention for use in
25 medical therapy or diagnosis.

The invention also provides the use of a complex of the invention to prepare a medicament useful for suppressing bone marrow in a mammal, for treating cancer in a mammal, for treating bone pain in a mammal, or for treating a bone related disease in a mammal.

30 The invention also provides intermediates useful for preparing complexes of the invention (e.g., compounds of formula I), as well as processes

useful for preparing complexes of the invention and for preparing compounds of formula I.

The present invention also provides compositions comprising a complex of the invention combined with an effective stabilizing amount of ascorbic acid or other stabilizing agent (e.g. gentisic acid) buffered to pH 7-8, as well as methods for preparing the compositions. The ascorbic acid maintains the radionuclide complex stability and reduces the amount of free radionuclide delivered *in vivo*. For example, ascorbic acid may be present in the unit dosage forms useful in the practice of the present invention at about 35-75 mg/ml of composition. Stabilization unexpectedly inhibits radiolytic degradation of the complexes, and thus allows distribution to hospitals at high levels of purity, with high levels of the radionuclide.

Brief Description of the Figures

15	FIG. 1	illustrates representative compounds of formula (I)
	FIG. 2	illustrates representative compounds of formula (I)
	FIG. 3	illustrates representative compounds of formula (I)
	FIG. 4-9	illustrate the synthesis of representative compounds of formula (I), and intermediates useful for preparing
20		compounds of formula (I).

Detailed Description

The following definitions are used, unless otherwise described: halo is fluoro, chloro, bromo, or iodo. Alkyl denotes both straight and branched groups; but reference to an individual radical such as "propyl" embraces only the straight chain radical, a branched chain isomer such as "isopropyl" being specifically referred to. Aryl denotes a phenyl radical or an ortho-fused bicyclic carbocyclic radical having about nine to ten ring atoms in which at least one ring is aromatic.

It will be appreciated by those skilled in the art that compounds of the invention having a chiral center may exist in and be isolated in optically

active and racemic forms. Some compounds may exhibit polymorphism. It is to be understood that the present invention encompasses any racemic, optically-active, polymorphic, or stereoisomeric form, or mixtures thereof, of a compound of the invention, which possess the useful properties described herein, it being
5 well known in the art how to prepare optically active forms (for example, by resolution of the racemic form by recrystallization techniques, by synthesis from optically-active starting materials, by chiral synthesis, or by chromatographic separation using a chiral stationary phase).

The term “bone marrow restoration” includes partial or complete
10 regeneration or augmentation of the bone marrow by marrow transplantation or stem cell transplantation and/or stimulation of bone marrow regeneration by administration of growth factors such as cytokines, glycoproteins and the like.

The term “bone marrow transplant (BMT)” includes autologous, allogenic, xenogenic marrow transplantation and stem cell transplantation.

15 The term “calcified tissue” includes bone as well as other tissue that may be undergoing pathological calcification.

The term “disease” includes pathologies and deleterious conditions, such as inflammatory responses and genetic disorders.

The term “mammal” means a warm blooded mammal, including
20 humans, and is meant to encompass mammals in need of bone marrow suppression, especially humans; thus in some instances the term “patient” or “subject” is alternatively used for mammal.

The term “suppressing bone marrow” includes partial or essentially total eradication (ablation or myeloablation) of the bone marrow, in
25 particular, a temporary or permanent reduction of the hemopoietic stem cell population.

The present complexes and methods can also be employed to treat pathologies other than cancer associated with (at or near) mammalian bone, that can be ameliorated by partial bone marrow suppression or by complete bone
30 marrow ablation followed by bone marrow transplantation. Thus, the term “bone related diseases” includes but is not limited to, immunological disorders such as

- autoimmune diseases, e.g., Crohn's disease, rheumatoid arthritis or multiple sclerosis; metabolic diseases, such as osteoporosis or osteopenia; infections and infectious disease, inflammatory diseases such as osteomyelitis or Paget's disease; hematopoietic disorders, and conditions treatable with stem cell
- 5 transplantation, with or without gene therapy, that utilize bone marrow ablation, such as sickle cell anemia and lysosomal and peroxisomal storage diseases.

Complexes

- Typically, the radionuclide complex is taken up preferentially by
- 10 bone so that it is possible to deliver a bone marrow suppressing dose of radiation to the bone marrow. The radionuclide also should be cleared rapidly from the blood. The radiation exposure is reported using the Grey scale (Gy). The amount of radioactivity required to deliver a desired dose to marrow can be determined using a diagnostic dose of about 1200-2000 MBq (about 30 mCi to
- 15 about 50 mCi) of the radionuclide/ligand. A determination of the doses of radiation delivered by the present complexes can be made using the methodologies of Bardies, M. and M. Myers (1996); "Computational methods in radionuclide dosimetry." Physics in Medicine and Biology **41**: 1941-1955; Bayouth, J. (1993); A Dosimetric Study of Radionuclide Therapy for Bone
- 20 Marrow Ablation. Radiation Physics, Houston, University of Texas - Houston Graduate School of Biomedical Science: 111; Beddoe, A. H., P. J. Darley, *et al.* (1976); "Measurements of trabecular bone structure in man." Physics in Medicine & Biology **21**(4): 589-607; Bigler, R. and H. Woodard (1976); "Skeletal Distribution of Mineralized Bone Tissue in Humans." Health Physics
- 25 **31**(9): 213-218; Champlin, R. and R. Gale (1987); "Bone marrow transplantation for acute leukemia: recent advances and comparison with alternative therapies." Semin. Hematol **24**: 55-67; Champlin, R. E. and R. P. Gale (1984); "Role of bone marrow transplantation in the treatment of hematologic malignancies and solid tumors: critical review of syngeneic, autologous, and allogeneic
- 30 transplants." Cancer Treatment Reports **68**(1): 145-61; Eckerman, K. and M. Stabin (1994); "Dose conversion factors for marrow and bone by skeletal

- regions; “Journal of Nuclear Medicine(35): 112P; Hiu, T. E. and J. W. Poston (1987). A model of the circulating blood for use in radiation dose calculations. Proceedings of International Conference on Radiation Dosimetry and Safety, Taipei, Taiwan, American Nuclear Society; I.C.R.P (1973). Report of the task
 5 group on reference man: anatomical, physiological and metabolic characteristics. New York, Pergamon Press; Loevinger, R. L., T. F. Budinger, *et al.* (1991); MIRD Primer for Absorbed Dose Calculations. New York, Society of Nuclear Medicine; Spiers, F. W., A. H. Beddoe, *et al.* (1981). “Mean skeletal dose factors for beta-particle emitters in human bone. Part II: surface-seeking radionuclides.”
 10 British Journal of Radiology **54**(642): 500-4; Thomas, S. R., M. G. Stabin, *et al.* (1992); “MIRD Pamphlet No. 14: a dynamic urinary bladder model for radiation dose calculations [published erratum appears in J. Nucl. Med. 1994 Jan;35(1):73],” Journal of Nuclear Medicine **33**(5): 783-802.

It is important that the half-life of the complexed radionuclides be
 15 sufficiently long to allow for localization of the complex in the bone tissue, while still retaining sufficient radioactivity to accomplish bone marrow suppression or eradication. Generally, it is preferred to use a radionuclide complex that results in rapid biolocalization of the radionuclide in the bone tissue so as to achieve bone marrow irradiation quickly. It is also beneficial to
 20 use a radionuclide having sufficient beta energy, while having a relatively short half-life so that after bone marrow irradiation is achieved, it is possible to proceed with bone marrow or stem cell transplantation as soon as possible, in order to enhance the prospects of bone marrow engraftment and patient recovery.

Detectable or therapeutic radionuclides (i.e., radioisotopes or
 25 paramagnetic atoms) suitable for incorporation in the complexes of the invention include Antimony-124, Antimony-125, Arsenic-74, Arsenic-77, Barium-103, Barium-140, Beryllium-7, Bismuth-206, Bismuth-207, Bismuth-212, Cadmium-109, Cadmium-115m, Calcium-45, Cerium-139, Cerium-141, Cerium-144, Cesium-137, Chromium-51, Cobalt-55, Cobalt-56, Cobalt-57, Cobalt-58,
 30 Cobalt-60, Cobalt-64, Copper-67, Erbium-169, Europium-152, Gallium-64, Gallium-68, Gadolinium-153, Gadolinium-157 Gadolinium-159, Gold-195,

Gold-198, Gold-199, Hafnium-175, Hafnium-175-181, Holmium-166, Indium-110, Indium-111, Iridium-192, Iron-55, Iron-59, Krypton-85, Lead-203, Lead-210, Lutetium-177, Manganese-54, Mercury-197, Mercury-203, Molybdenum-99, Neodymium-147, Neptunium-237, Nickel-63, Niobium-95, Osmium-185 +
 5 191, Osmium-193, Palladium-103, Platinum-195m, Praseodymium-143, Promethium-147, Promethium-149, Protactinium-233, Radium-226, Rhenium-186, Rhenium-188, Rubidium-86, Ruthenium-97, Ruthenium-103, Ruthenium-105, Ruthenium-106, Samarium-153, Scandium-44, Scandium-46, Selenium-75, Silver-110m, Silver-111, Sodium-22, Strontium-85, Strontium-89, Strontium-90,
 10 Sulfur-35, Tantalum-182, Technetium-99m, Tellurium-125, Tellurium-132, Thallium-204, Thorium-200, Thorium-228, Thorium-232, Thallium-170, Tin-113, Tin-114, Tin-117m, Titanium-44, Tungsten-185, Vanadium-48, Vanadium-49, Ytterbium-169, Ytterbium-175, Yttrium-86, Yttrium-88, Yttrium-90, Yttrium-91, Zinc-65, and Zirconium-95.

15 The term “detectable radionuclide” includes any suitable metallic radionuclide or paramagnetic atom that can be detected in a diagnostic procedure *in vivo* or *in vitro*.

 The term “therapeutic radionuclide” includes any metallic radionuclide that is suitable to provide a therapeutic effect (e.g., bone marrow
 20 suppression or ablation, reducing bone pain, treating bone cancer, or treating a bone related disease), in a mammal. Such radionuclides should be capable of delivering a high enough localized ionization density to achieve a desired therapeutic result (e.g., alleviate pain, inhibit tumor growth, cause regression of a tumor, and/or destroy a tumor).

25 Radionuclides suitable for bone marrow suppression typically exhibit beta energy >0.5 MeV, preferably >1 MeV with an effective half-life of about < 5 days, preferably < 3 days. Certain radionuclides such as Strontium-89 have been demonstrated, when selectively deposited in bone, to suppress bone marrow. [See, for example, Y. Shibata *et al.*, J. Leukocyte Biol. 38(6), 659-669
 30 (Dec. 1985).] However, this compound is not clinically useful for bone marrow

replacement since the long half-life of Strontium-89 (50 days) prevents transplantation of the new marrow for an unacceptable time.

The term "amino acid," comprises the residues of the natural amino acids (e.g., Ala, Arg, Asn, Asp, Cys, Glu, Gln, Gly, His, Hyl, Hyp, Ile, 5 Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val) in D or L form, as well as unnatural amino acids (e.g., phosphoserine, phosphothreonine, phosphotyrosine, hydroxyproline, gamma-carboxyglutamate; hippuric acid, octahydroindole-2-carboxylic acid, statine, 1,2,3,4,-tetrahydroisoquinoline-3-carboxylic acid, penicillamine, ornithine, citruline, α -methyl-alanine, para-benzoylphenylalanine, 10 phenylglycine, propargylglycine, sarcosine, and tert-butylglycine). The term also comprises natural and unnatural amino acids bearing a conventional amino protecting group (e.g., acetyl or benzyloxycarbonyl), as well as natural and unnatural amino acids protected at the carboxy terminus (e.g., as a (C₁-C₆)alkyl, phenyl or benzyl ester or amide). Other suitable amino and carboxy protecting 15 groups are known to those skilled in the art (See for example, T.W. Greene, *Protecting Groups In Organic Synthesis*, 2nd edition; Wiley: New York, 1991, and references cited therein). The term non-lipophilic amino acid comprises the natural amino acids Gly, Ser, Thr, Cys, Met, Asn, Gln, Tyr, Lys, Arg, His, Asp, and Glu in D and L forms, as well as unnatural amino acids of comparable 20 degree of hydrophilicity--i.e., those lacking a hydrophobic side chain. An amino acid can be linked to the remainder of a compound of formula I through the carboxy terminus, the amino terminus, or through any other convenient point of attachment, such as, for example, through the sulfur of cysteine. The term amino acid also comprises natural and unnatural amino acids substituted on 25 nitrogen with (C₁-C₁₀)alkyl.

The term "saccharide" includes monosaccharides, disaccharides, trisaccharides and polysaccharides. The term includes glucose, sucrose fructose and ribose, as well as deoxy sugars such as deoxyribose and the like. Saccharide derivatives can conveniently be prepared as described in International Patent 30 Applications Publication Numbers WO 96/34005 and 97/03995. A saccharide

can conveniently be linked to the remainder of a compound of formula I through an ether bond.

The term "peptide" describes a sequence of 2 to 25 amino acids (e.g., as defined hereinabove) or peptidyl residues. The sequence may be linear or cyclic. For example, a cyclic peptide can be prepared or may result from the formation of disulfide bridges between two cysteine residues in a sequence. A peptide can be linked to the remainder of a compound of formula I through the carboxy terminus, the amino terminus, or through any other convenient point of attachment, such as, for example, through the sulfur of a cysteine. Preferably a peptide comprises 3 to 25, or 5 to 21 amino acids. Peptide derivatives can be prepared as disclosed in U.S. Patent Numbers 4,612,302; 4,853,371; and 4,684,620, or as described in the Examples hereinbelow. Peptide sequences specifically recited herein are written with the amino terminus on the left and the carboxy terminus on the right. Preferred peptides comprise primarily non-lipophilic amino acid residues (e.g., Gly, Ser, Thr, Cys, Met, Asn, Gln, Tyr, Lys, Arg, His, Asp, and Glu). Preferred peptides comprise one or more amino acids substituted on nitrogen with (C₁-C₁₀)alkyl.

For the diagnostic methods of the invention, the complexes of the invention can be detected using any imaging technique known in the art that is suitable for detecting the metal in the complex. Suitable imaging techniques for detecting complexes of the invention include PET, SPECT, and MRI.

The complexes of the invention are particularly useful for treating primary tumors, where the skeletal system is the first site of involvement, invasive tumors where the primary tumor invades the skeletal system or other tissue tumors which calcify, and metastatic bone cancer where a neoplasm spreads from other primary sites, e.g., prostate or breast, into the skeletal system.

The structure of the linker group Y is not critical, provided the complex of the invention comprising the linker group is suitable for its intended use as an imaging agent or as a therapeutic agent, and provided the linker group can be substituted with phosphono groups as provided for in the compounds of formula I. Therapeutic complexes comprising the linker group should be

suitably stable and suitably non-toxic to allow for the complex to be administered to a mammal.

Specific and preferred values listed below for radicals, substituents, and ranges, are for illustration only; they do not exclude other
 5 defined values or other values within defined ranges for the radicals and substituents.

Specifically, (C₁-C₁₀)alkyl can be methyl, ethyl, propyl, isopropyl, butyl, iso-butyl, sec-butyl, pentyl, 3-pentyl, hexyl, heptyl, octyl, nonyl, or decyl; (C₁-C₄)alkyl can be methyl, ethyl, propyl, isopropyl, butyl, iso-butyl, or sec-
 10 butyl; and aryl can be phenyl, indenyl, or naphthyl.

A specific value for R¹ is (C₁-C₄)alkyl, substituted with carboxy.

Another specific value for R₁ is carboxymethyl or 2--carboxyethyl.

A specific value for n is 2 or 3.

15 Specifically, the linker group can separate the phosphono groups from the remainder of a compound of formula (I) by about 5 angstroms to about 100 angstroms. More specifically, the linker group can separate the phosphono groups from the remainder of a compound of formula (I) by about 10 angstroms to about 50 angstroms.

20 Specifically, the linker group can be an amino acid, a peptide, a saccharide, or a divalent (C₁-C₁₀)alkyl chain, optionally comprising one or more (e.g., 1, 2, 3, or 4) non-peroxide oxy (-O-), -N(R_d)-, or divalent aryl within the chain or at the terminus of the chain, which chain is optionally substituted on carbon with one or more (e.g., 1, 2, 3, or 4) oxo (=O), thioxo (=S), or hydroxy,
 25 wherein R_d is hydrogen or (C₁-C₄)alkyl.

Specifically, when the linker group is a peptide, it can comprise from 2 to about 25 amino acids residues.

Specifically, the linker group can be a divalent (C₁-C₁₀)alkyl chain, comprising one or more (e.g., 1, 2, 3, or 4) non-peroxide oxy (-O-), -
 30 N(R_d)-, or divalent aryl within the chain or at the terminus of the chain, which

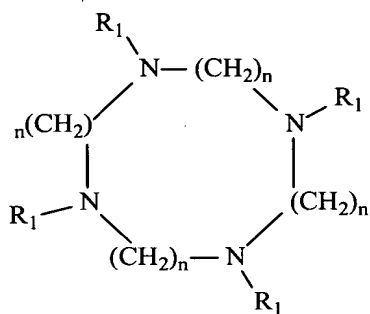
chain is optionally substituted on carbon with one or more (e.g., 1, 2, 3, or 4) oxo (=O), thioxo (=S), or hydroxy, wherein R_d is hydrogen or (C_1-C_4) alkyl.

Specifically, the linker group can be a divalent (C_1-C_{10}) alkyl chain, optionally comprising one or more (e.g., 1, 2, 3, or 4) non-peroxide oxy (-O-), -N(R_d)-, or divalent aryl within the chain or at the terminus of the chain,
 5 which chain is substituted on carbon with one or more (e.g., 1, 2, 3, or 4) oxo (=O), thioxo (=S), or hydroxy, wherein R_d is hydrogen or (C_1-C_4) alkyl.

Specifically, the linker group can be a divalent (C_1-C_{10}) alkyl chain comprising one or more (e.g., 1, 2, 3, or 4) non-peroxide oxy (-O-), -N(R_d)-, or
 10 divalent aryl within the chain or at the terminus of the chain, which chain is substituted on carbon with one or more (e.g., 1, 2, 3, or 4) oxo (=O), thioxo (=S), or hydroxy, wherein R_d is hydrogen or (C_1-C_4) alkyl.

Specifically, the group $-Y(PO_3H_2)_m$ can be 4-[2-(Bis-phosphonomethyl-amino)-acetyl-amino]-benzyl; 4-[4-(Bis-phosphonomethyl-carbamoyl)-butyrylamino]-benzyl; 4-(3,3-Bis-phosphono-propionylamino)-benzyl; 4-[4-(3-hydroxy-3,3-bis-phosphono-propylcarbamoyl)-butyrylamino]-benzyl; 4-(3-[2-(Bis-phosphonomethyl-amino)-acetyl-amino]-2-[[2-(bis-phosphonomethyl-amino)-acetyl-amino]-methyl]-propionylamino)-benzyl; 4-(4-{Bis-[(bis-phosphonomethyl-carbamoyl)-methyl]-carbamoyl}-butyrylamino)-benzyl; 4-{3-(3,3-Bis-phosphono-propionylamino)-2-[(3,3-bis-phosphono-propionylamino)-methyl]-[propionylamino]-benzyl; 4-(4-{Bis-[(3-hydroxy-3,3-bis-phosphono-propylcarbamoyl)-methyl]-carbamoyl}-butyrylamino)-benzyl; 4-{4-[(Bis-phosphono-methyl)-carbamoyl]-butyrylamino}-benzyl; or 4-[4-(Bis-[[bis-phosphono-methyl)-carbamoyl]-methyl]-carbamoyl)-butyrylamino]-benzyl.
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A specific complex of the invention is a complex wherein the compound of formula (I) is a compound of formula II:

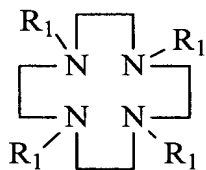


(II)

wherein:

- each R_1 is independently hydrogen or (C_1-C_4) alkyl, optionally substituted with carboxy (COOH); and each n is independently 2, 3, or 4; wherein the compound of formula (II) is substituted on one or more (e.g. 1, 2, 3, or 4) carbons other than a carbon of R_1 with one or more (e.g., 1, 2, 3, or 4) groups $-Y(PO_3H_2)_m$; wherein Y is a linker group; and m is 1, 2, 3, 4, 5, or 6; or a pharmaceutically acceptable salt thereof.

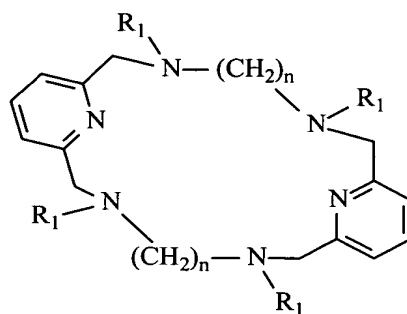
Another specific complex of the invention is a complex wherein the compound of formula I is a compound of formula III:



(III)

- 10 wherein each R_1 is independently hydrogen or (C_1-C_4) alkyl, optionally substituted with carboxy (COOH); and wherein the ring is substituted on carbon with one or more (e.g., 1, 2, 3, or 4) groups $-Y(PO_3H_2)_m$; wherein Y is a linker group; and m is 1, 2, 3, 4, 5, or 6.

- 15 Another specific complex of the invention is a complex wherein the compound of formula (I) is a compound of formula (IV):



(IV)

wherein:

each R_1 is independently hydrogen or (C_1-C_4) alkyl, optionally substituted with carboxy (COOH); and each n is independently 2, 3, or 4; wherein the compound of formula IV is substituted on one or more (e.g. 1, 2, 3, or 4) carbons other than a carbon of R_1 with one or more (e.g., 1, 2, 3, or 4) groups $-Y(PO_3H_2)_m$; wherein Y is a linker group; and m is 1, 2, 3, 4, 5, or 6; or a pharmaceutically acceptable salt thereof.

Another specific complex of the invention is a complex comprising a compound of formula (I), (II), (III), or (IV) as described herein, wherein each R_1 is independently (C_1-C_4) alkyl, substituted with carboxy (COOH); which compound is substituted with a group $-Y(PO_3H_2)_m$; wherein Y is a linker group; and m is 1, 2, 3, 4, 5, or 6.

A specific complex of the invention is a complex which comprises a detectable radionuclide.

A specific complex of the invention is a complex which comprises a therapeutic radionuclide.

A specific detectable radionuclide is Technetium-99m, Ruthenium-97, Indium-111, Gallium-67 or -68, or Lead-203.

A specific therapeutic radionuclide is Gadolinium-159, Holmium-166*, Lutetium-177, Samarium-153, Yttrium-90*, Ytterbium-175, Rhenium-186* or -188*, Copper-64 or -67, Gold-198 or -199, Bismuth-212, Scandium-46, -47, or -48, Gallium-72 or -73, Ruthenium-97, Palladium-100 or -109, Rhodium-101m or -105, Rhenium-189, Radium-212, or Lead-212

Specific radionuclides suitable for bone marrow suppression are Arsenic-77, Molybdenum-99, Ruthenium-105, Lutetium-177, Cadmium-115, Antimony-122, Promethium-149, Osmium-193, Gold-198, or Thorium-200. A more specific radionuclide suitable for bone marrow suppression is Samarium-
5 153, Yttrium-90, Gadolinium-159, Rhenium-186 or -188, or Holmium-166. A preferred radionuclide for bone marrow suppression is Holmium-166, which emits high energy beta particles and imageable gamma radiation (80 KeV, 6%) and exhibits a half-life of 26.8 hr.

A specific therapeutic radionuclide is Holmium-166, Yttrium-90,
10 Samarium-153, or Gadolinium-159.

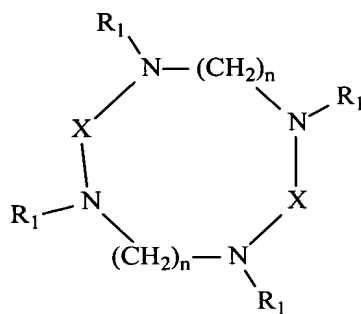
A more specific therapeutic radionuclide is Holmium-166.

Processes and intermediates useful for preparing compounds of formula I and complexes of the invention are provided as further embodiments of the invention and are illustrated by the following procedures in which the
15 meanings of the generic radicals are as given above unless otherwise qualified.

Radionuclide Complex Preparation

The radionuclide and the compound of formula I can be combined under any conditions that allow the two to form a complex. Generally, mixing in
20 water at a controlled pH (the choice of pH is dependent upon the choice of ligand and radionuclide) is all that is required for formation of the complex. Following complexation, it may be necessary to adjust the pH to a level that is suitable for administration. The complex is typically formed by chelation of the radionuclide by an electron donor group or groups, which results in a relatively stable
25 radionuclide complex, e.g., stable to the disassociation of the radionuclide from the ligand. Accordingly, the invention provides a method for preparing a complex of the invention comprising reacting a corresponding compound of formula I as described herein with a detectable or therapeutic radionuclide to provide the complex.

30 The invention also provides an intermediate compound of formula I:



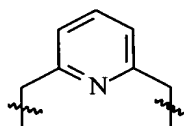
(I)

wherein:

each R₁ is independently hydrogen or (C₁-C₄)alkyl, optionally substituted with carboxy (COOH);

each X is independently (CH₂)_n or

5



and each n is independently 2, 3, or 4;

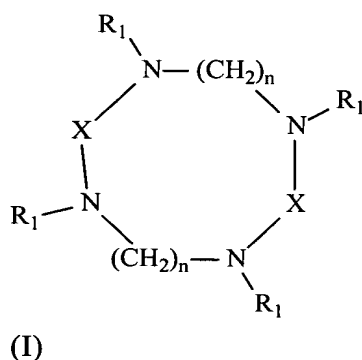
wherein the compound of formula I is substituted on one or more (e.g. 1, 2, 3, or 4) carbons other than a carbon of R₁ with one or more (e.g., 1, 2, 3, or 4) groups -Y(PO₃H₂)_m; wherein Y is a linker group; and m is 1, 2, 3, 4, 5, or 6. Such an intermediate compound of formula (I) is useful for preparing a complex of the invention.

The invention also provides an intermediate compound of formula II, III, or IV as described herein.

A compound of formula I that comprises one or more carboxy groups (e.g., in R₁) can conveniently be prepared by deprotecting a corresponding compound wherein the carboxy groups are protected by a suitable carboxy protecting group (e.g., as a (C₁-C₆)alkyl or benzyl ester). Suitable protecting groups for carboxy groups as well as conditions suitable for their removal are well known in the art. For example, see Greene, T.W.; Wutz, P.G.M. "Protecting Groups In Organic Synthesis" second edition, 1991, New

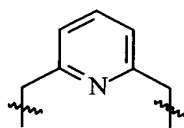
York, John Wiley & sons, Inc. Thus, the invention provides a method for preparing a compound of formula I wherein one or more of R_1 comprises a carboxy group comprising deprotecting a corresponding compound wherein one or more of the R_1 carboxy groups are protected by a suitable carboxy protecting group to provide the compound of formula I.

Accordingly, the invention also provides an intermediate compound of formula I:



wherein:

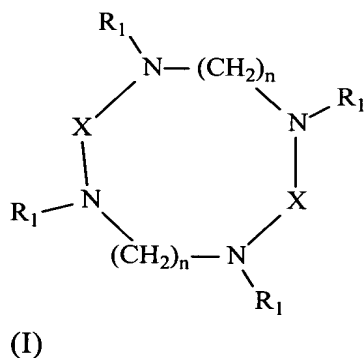
- each R_1 is independently (C_1-C_4) alkyl, substituted with COOR; R is a suitable carboxy protecting group (e.g., (C_1-C_4) alkyl or benzyl);
 each X is independently $(CH_2)_n$ or



- and each n is independently 2, 3, or 4;
 wherein the compound of formula I is substituted on one or more (e.g. 1, 2, 3, or 4) carbons other than a carbon of R_1 with one or more (e.g., 1, 2, 3, or 4) groups $-Y(PO_3H_2)_m$; wherein Y is a linker group; and m is 1, 2, 3, 4, 5, or 6.
- The invention also provides carboxy protected intermediate compounds of formula II, III, and IV as described herein wherein each R_1 is

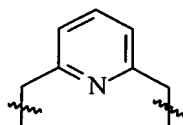
independently (C₁-C₄)alkyl, substituted with COOR; R is a suitable carboxy protecting group (e.g., (C₁-C₄)alkyl or benzyl).

- A compound of formula I that is substituted with one or more (e.g., 1, 2, 3, or 4) groups -Y(PO₃H₂)_m can conveniently be prepared by
- 5 deprotecting a corresponding compound that is substituted with one or more (e.g., 1, 2, 3, or 4) groups -Y(PO₃R₂)_m wherein R is a suitable phosphonic acid protecting group (e.g., a (C₁-C₆alkyl) or benzyl ester). Suitable protecting groups for phosphonic acid groups as well as conditions suitable for their removal are well known in the art. Thus, the invention provides a method for preparing a
- 10 compound of formula I that is substituted with one or more (e.g., 1, 2, 3, or 4) groups -Y(PO₃H₂)_m comprising deprotecting a corresponding compound that is substituted with one or more (e.g., 1, 2, 3, or 4) groups -Y(PO₃R₂)_m wherein each R is a suitable phosphonic acid protecting group(s) (e.g., a (C₁-C₆alkyl) or benzyl ester), to provide the compound of formula I.
- 15 The invention also provides an intermediate compound of formula I:



wherein:

- each R₁ is independently (C₁-C₄)alkyl, substituted with COOR; R
- 20 is a suitable carboxy protecting group (e.g., (C₁-C₄)alkyl or benzyl);
- each X is independently (CH₂)_n or



and each n is independently 2, 3, or 4;

wherein the compound of formula I is substituted on one or more (e.g. 1, 2, 3, or 4) carbons other than a carbon of R_1 with one or more (e.g., 1, 2, 3, or 4) groups $-Y(PO_3R_2)_m$; wherein Y is a linker group; each R is a suitable protecting group (e.g., (C_1-C_4) alkyl or benzyl); and m is 1, 2, 3, 4, 5, or 6.

The invention also provides phosphono protected intermediate compounds of formula II, III, and IV as described herein that are substituted on one or more (e.g. 1, 2, 3, or 4) carbons other than a carbon of R_1 with one or more (e.g., 1, 2, 3, or 4) groups $-Y(PO_3R_2)_m$; wherein Y is a linker group; each R is a suitable protecting group (e.g., (C_1-C_4) alkyl or benzyl); and m is 1, 2, 3, 4, 5, or 6.

Radionuclide Preparation

The respective radionuclides can be obtained using procedures well known in the art. Typically, the desired radionuclide can be prepared by irradiating an appropriate target, such as a metal, metal oxide, or salt. Another method of obtaining radionuclides is by bombarding nuclides with particles in a linear accelerator or cyclotron. Yet another way of obtaining radionuclides is to isolate them from fission product mixtures. The method of obtaining the radionuclide is not critical.

Stabilizing Agents

A pharmaceutically acceptable means of protecting the complexes of the invention from radiolytic decay can be employed. Preferred radioprotectants of the present invention are anti-oxidants, compounds that either reduce the number or the activity of oxidizing radicals. Exemplary

radioprotectants that can be employed in the practice of the present invention are ascorbic acid, gentisic acid, nicotinic acid, ascorbyl palmitate, HOP(O)H_2 , monthioglycerol, sodium formaldehyde sulfoxylate, $\text{Na}_2\text{S}_2\text{O}_5$, $\text{Na}_2\text{S}_2\text{O}_3$, SO_2 , or a reducing agent combined with BHA, BHT, pyrogallate or tocopherol and the like. Ascorbic acid is the preferred radioprotectant for use in the practice of the present invention, and can be used at about 35-75 mg/mL of liquid composition.

The formulations of the present invention can be in solid or liquid form, containing the active radionuclide complexed with the ligand. These formulations can be in kit form such that the chelator and radionuclide are mixed at the appropriate time prior to use in a suitable liquid carrier. Whether premixed or as a kit, the formulations usually require a pharmaceutically acceptable carrier.

Pharmaceutical Dosage Forms

The pharmaceutical dosage forms suitable for injection or infusion can include sterile solutions, dispersions, emulsions, or microemulsions, comprising the active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in protective matrices such as nanoparticles or microparticles. In all cases, the ultimate dosage form should be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, phosphonate or carbonate esters, DMSO, and suitable mixtures thereof. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, benzyl alcohol, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the

compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Injectable suspensions as compositions of the present invention can comprise a liquid suspending medium, with or without adjuvants, as a carrier. The suspending medium can be, for example, aqueous polyvinylpyrrolidone, inert oils such as vegetable oils or highly refined mineral oils, or aqueous carboxymethylcellulose solutions. If necessary to keep the complex in suspension, suitable physiologically acceptable adjuvants can be chosen from among thickeners such as, for example, carboxymethylcellulose, polyvinylpyrrolidone, gelatin, and the alginates. Many surfactants are also useful as suspending agents, for example, lecithin, alkylphenol, polyethylene oxide adducts, naphthalenesulfonates, alkylbenzenesulfonates, and the polyoxyethylene sorbitan esters. Many substances which effect the hydrophobicity, density, and surface tension of the liquid suspension medium can assist in making injectable suspensions in individual cases. For example, silicone antifoams, sorbitol, and sugars are all useful suspending agents.

The “bone-marrow suppressing amount” or other effective therapeutic amount of radionuclide composition administered to achieve bone marrow suppression will vary according to factors such as the age, weight and health of the patient, the disease state being treated, the treatment regimen selected, as well as the nature of the particular radionuclide composition to be administered. For example, less activity will be needed for radionuclides with longer half lives. The energy of the emissions will also be a factor in determining the amount of activity necessary. The preferred range of activity per dose is from about 250 to about 3000 megabecquerels per kilogram of body weight of said mammal. A more preferred dosage delivers from about 750 to about 2500 megabecquerels per kilogram of body weight of said mammal. The most preferred dosage can deliver from about 1000 to about 2000 megabecquerels per kilogram of body weight of said mammal. The effective amount will typically be administered, generally by administration into the bloodstream, in a single or multi-dose infusion.

The radionuclide compositions employed in the method of the present invention are capable of delivering a significant portion, preferably greater than about 15%, of the radioactivity present in the composition to bone tissue while not deleteriously affecting non-target soft tissues. Therefore, for those disease states where the treatment regimen requires bone marrow suppression, the present invention is particularly advantageous since it provides a means of achieving selective reduction in the hemopoietic cell population without having to resort to total body irradiation (TBI), thus resulting in minimal damage to non-target tissues. Furthermore, because there is a reduction in the radiation dose delivered to non-target tissues (as compared to the use of total body irradiation alone), the present invention offers the opportunity to use the same or increased amounts of conventional chemotherapeutic regimens. In addition, if it is desirable to employ total body irradiation in conjunction with the bone marrow suppression method described herein, for example, in the treatment of leukemia, it can be possible to reduce the radiation dosage used for the total body irradiation and still obtain the same or higher level of reduction of leukemic cells.

As used herein, the term "single dosage" or "single dose" means that the total dosage of radionuclide complex is administered in one (1) or more doses (unit dosage forms) within a short period of time, e.g., less than about 24 hours. Preferably the doses will be administered within 12 hours, most preferably 8 hours. More preferably the doses will be administered within 4 hours. More preferably the doses will be administered within 2 hours. Most preferably the dose will be administered as a single infusion, or other unit dosage form.

Bone Marrow Transplantation and Restoration

The general techniques of bone marrow transplantation are well known in the art, see for example, F. R. Appelbaum et al., "The Role of Marrow Transplantation in the Treatment of Leukemia", (pp. 229-262), C. D. Bloomfield (ed.), Chronic and Acute Leukemias in Adults, 1985, Martinus Nijhoff

Publishers, Boston; E. D. Thomas, "Clinical Trials with Bone Marrow Transplantation", (pp. 239-253), Clinical Trials in Cancer Medicine, 1985, Academic Press, Inc.; E. D. Thomas, "Marrow Transplantation for Malignant Diseases", (pp. 517-531), Journal of Clinical Oncology, Vol. 1, No. 9 (Sept.)
5 1983; E. D. Thomas et al., "Marrow Transplantation for Thalassemia", (pp. 417-427), Annals New York Academy of Sciences, 445, 1985.

Under general or spinal anesthesia and using standard marrow aspiration needles, multiple aspirates are performed from the anterior and posterior iliac crests and, occasionally, the sternum of the donor. The marrow is
10 placed in heparinized tissue culture media and then, using metal screens, filtered to remove bony spicules and fat globules and to create a monocellular suspension. At the time of desired administration of the bone marrow, the marrow is infused intravenously, following which the marrow stem cells migrate to the marrow space, proliferate, and eventually restore normal hematopoiesis
15 and immune function. It is preferable to give as many bone marrow cells as possible to enhance the prospects of marrow engraftment. Following allogeneic transplant the patient usually receives some form of immunosuppression, such as by administration of methotrexate or cyclosporine, in an attempt to prevent or at least modify graft-versus-host disease.

20 A more preferred method for retrieving bone marrow stem cells involves harvesting these cells from the peripheral blood to increase the concentration of stem cells in the blood by techniques such as negative selection with antibodies specific for hematopoietic cell markers. Patients are pretreated with chemotherapy, or pretreated with a colony stimulating factor such as G-
25 CSF, GM-CSF, or SC-CSF. These cytokines are also used after TBI and marrow or stem cell transplant to enhance engraftment.

The use of high dose chemotherapy followed by stem cell support has become one of the most attractive therapeutic approaches in multiple myeloma since, in relation to conventional chemotherapy, it increases the
30 number of complete remissions (CR), duration of event free survival (EFS) and probably, overall survival (OS). In this setting of high dose chemotherapy, the

use of a complex of the invention to suppress (ablate) the marrow in order to potentially eradicate the malignant clone more effectively, requires stem cell support. With total marrow ablation using a complex of the invention a stem cell rescue is required using autologous stem cells collected prior to therapy. The
5 ability to give back the patients stem cells post ablative therapy helps to regenerate the lost hematopoiesis and thus protect the patient from potentially life-threatening complications.

Treatment of Cancer

10 A. Chemotherapeutic Agents

In the treatment of a patient having a cancer such as leukemia or multiple myeloma, the use of the radionuclide compositions described herein can reduce or eliminate the neoplastic cell population in the bone marrow. The phosphonate complexes of the invention also provide enhanced penetration of
15 bone and uptake of the radionuclide by neoplastic bone lesions, which represent areas of active bone matrix turnover. However, it will usually be necessary to administer one or more chemotherapeutic agents, such as melphalan, dimethyl busulfan, cytoxan and/or cyclophosphamide, to destroy the undesirable cells in locations other than the bone marrow or in sanctuaries within the bone marrow,
20 or to add to the effects of the radiation. The efficacy of cancer elimination can be enhanced by the use of protein synthesis inhibitors, in order to inhibit repair of damaged DNA in the cancer cells.

Chemotherapy can be given in standard doses in conjunction with the present method, or as higher than standard doses along with the present
25 invention, depending on the tolerance of the patient. In other instances in conjunction with the bone marrow suppression method of the present invention, it is often desirable to employ total body irradiation, with or without chemotherapeutic agents, as a treatment to reduce the malignant or neoplastic cell population, such as by delivering radiation to the patient from dual opposing
30 cobalt-60 sources. Those skilled in the art will know how to perform a Phase I

study determining the limits of tolerance of either the chemotherapy, irradiation or the dose of the present complexes or both.

Chemotherapeutic agents that are useful in practicing the present invention include but are not limited to adriamycin, ifosfamide, thiotepa, melphalan, methotrexate, mitoxantrone, estramustine, bleomycin, velban, taxanes, thalidomide, etoposide, phosphate, taxol, vincristine, dexamethasone, doxorubicin, busulfan, cytoxan, cyclophosphamide, bischloroethyl nitrosourea, cytosine arabinoaside, 6-thioguanine and the like. Preferred chemotherapeutic agents that are useful in practicing the present invention are adriamycin, ifosfamide, thiotepa, melphalan, methotrexate, mitoxantrone, estramustine, bleomycin, velban, taxanes, thalidomide, vincristine, dexamethasone, doxorubicin, busulfan and analogs thereof. For example, melphalan analogs are disclosed in U.S. Pat. Nos. 3,032,584 and 3,032,585 (see Merck Index (11th ed.) at page 914).

The term "chemotherapeutic agent" also includes anti-cancer agents, such as toxins, that are targeted to cancer cells by antibodies against cancer cell antigens. Such immunoconjugates are described in published PCT applications WO/97/00476 and WO/95/10940.

The mammals (patients) can also be treated with agents such as bisphosphonates, to counteract the hypercalcemia associated with certain tumors, such as lung cancers, multiple myeloma, renal cell carcinoma, bronchogenic carcinoma, breast cancer, lymphoma, and cancers of the head and neck. Pamidronate and alendronate disodium are preferred agents for treatment of this condition. It will be appreciated that the agents should be selected and used so as not to compete with the therapeutic agent for bone marrow uptake.

The mammals (patients) can be hydrated and premedicated with antiemetics to decrease nausea and vomiting that may be associated with suppression of bone marrow when practicing the present invention. The preferred antiemetics are those that reduce the irritation of the chemoreceptor trigger zone. Common regimens that are useful in practicing the present invention include serotonin 5-HT³ antagonists such as, for example, ondasteron,

granisetron, and the like; dopamine antagonists such as, for example, prochlorperazine, promethazine, droperidol, metoclopramide, and the like; antihistamines and anticholinergics such as, for example, diphenylhydramine, scopolamine, dimethylhydrinate, meclizine, and the like; corticosteroids such as, for example, dexamethasone and the like; and sedatives such as, for example, diazepam, lorazepam, and the like.

A wide variety of leukemias and solid tumors can be treated with the present complexes including bone-forming tumors, cartilage-forming tumors, fibrous and fibro-osseous tumors, leukemias such as chronic lymphocytic leukemia and myeloid leukemia, and metastatic tumors to the skeleton. These skeletal system tumors include, but are not limited to, sarcomas such as Ewing's sarcoma, osteochondroma, chondroma, chondroblastoma, chondromyxoid fibroma and chondrosarcoma, non-ossifying fibroma, fibrous histiocytoma, fibrosarcoma, liposarcoma, sarcoma of the periosteum, osteosarcoma, osteoma, osteoblastoma and giant cell tumor of the bone. Other tumors which can be treated include chordoma, adamantoma, hemangioendothelioma, hemangiopericytoma, myelomas, such as multiple myeloma, non-Hodgkin's lymphoma, Hodgkin's disease, breast cancer, prostate cancer, lung cancer, head and neck cancer, ovarian cancer, bladder cancer, liver cancer, pancreatic cancer, renal cell carcinoma, myelodysplastic syndrome, germ cell tumor, and neuroblastoma, particularly those cancers that have metastasized to the bone, attach to the bone, or that are associated with the skeletal system. Myeloproliferative disorders, including polycythemia vera, macroglobulinemia, megakaryocytic myelosis or malignant histiocytosis, can also be treated with the present complexes.

Thus, the invention provides a method for treating cancer comprising administering to a mammal in need of such therapy, a complex of the invention comprising a therapeutic radionuclide, in combination with a chemotherapeutic agent, a bisphosphonate, or an antiemetic. The invention also provides a pharmaceutical composition comprising a complex of the invention

and a chemotherapeutic agent, a bisphosphonate, or an antiemetics; in combination with a pharmaceutically acceptable carrier.

B. Adjunct Radiation Therapy

5 By careful aiming and regulation of dose, high-energy radiation can be used to destroy cancer cells in combination with the present radionuclide therapy. Radiation therapy (also referred to as radiotherapy, x-ray therapy, cobalt treatment, or irradiation) is presently either part of the treatment or the only treatment for about half of all cancer patients. This form of treatment is effective
10 only for those cancer cells within the area receiving the radiation (the field), which can encompass the entirety of the subject's body (total body irradiation or TBI) or can be localized, as in the exposure of a specific tumor site.

 Radiation may be used before surgery to shrink a cancerous tumor, after surgery to stop growth of any remaining cancer cells, or alone or
15 with anticancer drugs to destroy a malignant tumor. It is particularly effective when used to treat certain types of localized cancers such as malignant tumors of the lymph nodes or vocal cords.

 Radiation usually is not *per se* curative if the cancer cells have spread throughout the body or outside the area of radiation. It can be used even
20 if a cure is not probable because it can shrink tumors, which decreases the pressure and pain they cause, or it can stop their bleeding.

 Generally, radiation produces less physical disfigurement than radical surgery does, but it may produce severe side effects. These side effects are related to the damage x-rays do to normal tissue such as blood or bone
25 marrow. Side effects include irritated skin, swallowing difficulties, dry mouth, nausea, diarrhea, hair loss, and a loss of energy. How serious and extensive these side effects become depend on where and how much radiation is used.

 Use of the present radionuclide complexes can reduce or eliminate the need for total or targeted external radiation therapy, or can enhance
30 the total efficacy of a therapeutic regimen which normally employs TBI. Doses of TBI useful in the present method can deliver total irradiation of from about

750-1350 cGy, e.g., about 800-1000 to 1200 cGy. The total irradiation may be given in multiple fractions, i.e., 1-10 fractions; or in a single dose.

Thus, the invention provides a method for treating cancer comprising administering to a mammal in need of such therapy, a complex of the
5 invention, in combination with radiation therapy.

Treatment of Autoimmune Diseases and Immunosuppression

The methods and complexes of the invention are also useful to treat immunological disorders such as autoimmune diseases by immune
10 suppression due to temporary partial bone marrow suppression or by marrow purging in combination with marrow transplantation. However, those skilled in the art would recognize that the methods and compositions of the invention can also be used for general immunosuppression in combination with other immunosuppressive therapies. Currently, autoimmune diseases are treated by a
15 variety of nonspecific immunosuppressive drugs and steroids. One group of anti-inflammatory agents used in the treatment of autoimmune diseases is corticosteroids. Corticosteroids are synthetic versions of the body's hormone cortisone, which is produced in small amounts by the adrenal gland. Synthetically produced corticosteroids reduce inflammation and suppress the
20 immune system. The most commonly prescribed corticosteroids for use in treating autoimmune disorders are prednisone and dexamethasone.

Autoimmune disorders are sometimes treated with immunosuppressant drugs such as cytotoxic agents (e.g., methotrexate, azathioprine and cyclophosphamide). In addition, anti-malarials including
25 chloroquine and hydroxychloroquine can be used to suppress inflammation and the immune system in the treatment of autoimmune disorders. Autoimmune diseases can also be treated with nonsteroidal anti-inflammatory medications, such as aspirin, ibuprofen, naproxen, indomethacin, sulindac, etodolac and tolmetin. Gold salts have been used to treat autoimmune arthritis for over a half
30 a century, while recent advances in research have yielded new autoimmune arthritis therapies, such as COX-2 inhibitors. COX-2 inhibitors (or super-

aspirin) work to inhibit inflammation and pain without producing significant side effects. In addition, another class of agents that target aberrant cytokine production, such as anti-TNF (tumor necrosis factor) drugs, can also be used for the treatment for several types of arthritis including rheumatoid arthritis, lupus, 5 myositis, and scleroderma.

Furthermore, the methods and complexes of the invention could also be used alone or in combination with drugs that act more specifically on the immune system, for example, by blocking a particular hypersensitivity reaction. In addition, the complexes could be used in combination with intravenous 10 immunoglobulin therapy or other antibody-based therapies, a treatment, used for various immunological diseases to reduce circulating immune complexes, or specific T cell populations. For example, the present methods and complexes can be used as immunosuppressive agents to inhibit host rejection of transplanted cells, tissue or organs.

15 In order to increase the chance of the patient's recovery, it can be beneficial to employ materials, such as granulocyte macrophage colony stimulating factor (GM-CSF), or granulocyte colony stimulating factor (G-CSF), and IL-11 for thrombopoiesis to stimulate or enhance the regeneration and restoration of the bone marrow. It can also be beneficial to employ stem cell 20 growth factor, G-CSF and/or GM-CSF prior to therapy to trigger release of stem cells into the blood where they can be collected.

Thus, the invention provides a method for treating an immunological disorder comprising administering to a mammal in need of such therapy, a complex of the invention, in combination with another 25 immunosuppressant therapy or immunosuppressant drug. The invention also provides a pharmaceutical composition comprising a complex of the invention and an immunosuppressant drug; in combination with a pharmaceutically acceptable carrier.

Infections and Infectious Diseases

The methods and complexes of the invention are also effective to treat bacterial infections, fungal infections, and infectious diseases that localize to or around bone such as tuberculosis, bacterial osteomyelitis, fungal
5 osteomyelitis, and the like. Anti-fungal agents, and anti-bacterial agents often have poor penetration into the bone and sites enclosed by bone such as the bone marrow. In situations in which the patient is suffering from an infectious disease which has localized to the bone, the patient may be able to achieve a cure by the delivery of high doses of radiation to the bone.

10 Examples of agents useful in combination with targeted radiation in practicing the present invention include, but are not limited to antibiotic agents, e.g., antibacterial urinary tract agents; anti-infective agents, anti-parasitic agents and anti-fungal agents, including those disclosed in The Physician's Desk Reference, 50th Edition, 1996.

15 Useful antibiotic agents include systemic antibiotics, such as aminoglycosides, cephalosporins (e.g., first, second, and third generation), macrolides (e.g., erythromycins), monobactams, penicillins, quinolones, sulfonamides, and tetracyclines, including those disclosed in The Physician's Desk Reference, 50th Edition, 1996.

20 In addition, antibacterial agents include 2-isocephem and oxacephem derivatives disclosed in U.S. Patent No. 5,919,925; pyridonecarboxylic acid derivatives disclosed in U.S. Patent No. 5,910,498; water miscible esters of mono- and diglycerides disclosed in U.S. Patent No. 5,908,862; benzamide derivatives disclosed in U.S. Patent No. 5,891,890; 3-
25 ammoniopropenyl cephalosporin compounds disclosed in U.S. Patent No. 5,872,249; 6-O-substituted ketolides disclosed in U.S. Patent No. 5,866,549; benzopyran phenol derivatives disclosed in U.S. Patent No. 5,861,430; pyridine derivatives disclosed in U.S. Patent No. 5,859,032; 2-aminothiazole derivatives disclosed in U.S. Patent No. 5,856,347; penem ester derivatives disclosed in U.S.
30 Patent No. 5,830,889; lipodepsipeptides disclosed in U.S. Patent No. 5,830,855; dibenzimidazole derivatives disclosed in U.S. Patent No. 5,824,698;

alkylenediamine derivatives disclosed in U.S. Patent No. 5,814,634; organic solvent-soluble mucopolysaccharides disclosed in U.S. Patent No. 5,783,570; arylhydrazone derivatives disclosed in U.S. Patent No. 5,760,063; carbapenem compounds disclosed in U.S. Patent No. 5,756,725; N-acylpiperazine derivatives
5 disclosed in U.S. Patent No. 5,756,505; peptides disclosed in U.S. Patent No. 5,714,467; oxathiazines and their oxides disclosed in U.S. Patent No. 5,712,275; 5-amidomethyl alpha beta-saturated and -unsaturated 3-aryl butyrolactone compounds disclosed in U.S. Patent No. 5,708,169; halogenated benzene derivatives disclosed in U.S. Patent No. 5,919,438; sulfur-containing
10 heterocyclic compounds disclosed in U.S. Patent No. 5,888,526; and oral antibacterial agents disclosed in U.S. Patent No. 5,707,610.

Anti-parasitic agents include agents capable of killing arthropods (e.g., lice and scabies); helminths (e.g., ascaris, enterobius, hookworm, stronglyoids, trematodes, and trichuris); and protozoa (e.g., amebas, malaria,
15 toxoplasma, and trichomonas), including those disclosed in The Physician's Desk Reference, 50th Edition, 1996.

The methods and compositions of the invention are also effective to treat fungal infections that localize to or around bone such as fungal osteomyelitis and the like. The methods and compositions can also be used in
20 conjunction with antifungal agents known to be useful in the treatment of fungal infections. Antifungal agents include dermatological fungicides, topical fungicides, systemic fungicides, and vaginal fungicides, including those disclosed in The Physician's Desk Reference, 50th Edition, 1996.

In addition, antifungal agents include terpenes, sesquiterpenes
25 diterpenes, and triterpenes disclosed in U.S. Patent No. 5,917,084; sulfur-containing heterocyclic compounds disclosed in U.S. Patent No. 5,888,526; carbozamides disclosed in U.S. Patent No. 5,888,941; phyllosilicates disclosed in U.S. Patent No. 5,876,738; corynrcandin derivatives disclosed in U.S. Patent No. 5,863,773; sordaridin derivatives disclosed in U.S. Patent No. 5,854,280;
30 cyclohexapeptides disclosed in U.S. Patent No. 5,854,213; terpene compounds disclosed in U.S. Patent No. 5,849,956; agents derived from aspergillus

fumigatus disclosed in U.S. Patent No. 5,873,726; inula extracts disclosed in U.S. Patent No. 5,837,253; lipodepsipeptides disclosed in U.S. Patent No. 5,830,855; polypeptides disclosed in U.S. Patent No. 5,824,874; pyrimidone derivatives disclosed in U.S. Patent No. 5,807,854; agents from sporomella
5 minimizes disclosed in U.S. Patent No. 5,801,172; cyclic peptides disclosed in U.S. Patent No. 5,786,325; polypeptides disclosed in U.S. Patent No. 5,773,696; triazoles disclosed in U.S. Patent No. 5,773,443; fusacandins disclosed in U.S. Patent No. 5,773,421; terbenzimidazoles disclosed in U.S. Patent No. 5,770,617; and agents obtained from hormones disclosed in U.S. Patent No. 5,756,472.

10 Thus, the invention provides a method for treating an infection or an infectious disease comprising administering to a mammal in need of such therapy, a complex of the invention, in combination with an antibiotic agent, an anti-infective agent, an anti-parasitic agent, or an anti-fungal agent. The invention also provides a pharmaceutical composition comprising a complex of
15 the invention and an antibiotic agent, an anti-infective agent, an anti-parasitic agent, or an anti-fungal agent; in combination with a pharmaceutically acceptable carrier.

Pathologies Treatable by BMT or Stem Cell Replacement

20 The present methods can be useful to ablate bone marrow in treatment regimens intended to correct a variety of disorders by replacing “defective” hematopoietic cells, with “normal” autologous or allogeneic bone marrow or stem cells. This can be used in the treatment of diseases of red cells and bleeding disorders. These include hematopoietic genetic diseases such as
25 hemolytic anemias, i.e., sickle cell anemia or thalassemia. Other such disorders include various anemias, polycythemia, thrombocytopenia, and bleeding disorders related to defective platelet function or abnormalities in clotting factors.

 Hematopoietic stem cell transplantation from normal donor has
30 been reported to be effective to treat lysosomal and peroxisomal storage diseases, such as globoid cell leukodystrophy, metachromatic leukodystrophy,

adrenoleukodystrophy, mannosidosis, flucosidosis, aspartylglucosaminuria; Harder, Maroteaux-Lamy and Sly Syndromes and Gaucher disease type III. W. Krivit et al., Curr. Opin. Neurol., 12, 167 (1999).

5 Gene Therapy

The present method can also be employed as part of gene therapy that involves implantation of genetically engineered stem cells, to correct genetic defects, following bone marrow ablation. For example, a subject's own stem cells can be "normalized" by introduction of a vector comprising a gene that will
10 effectively counteract the defective gene or replace the missing one. See, D.B. Kohn, Curr. Opinion in Pediatr., 7, 56 (1995).

Bone marrow suppression, followed by administration of genetically engineered (transformed) stem cells, can be used, for example, in the treatment of cancer in a human by inserting exogenous genes into human
15 primary cells, such as, for example, stem cells, which specifically "target" mature blood cells to a tumor. Preferably, the stem cells have been removed from a cancer patient and expanded in culture. Genes that enhance the anti-tumor effects of the mature cells can also be employed. The blood cells can be expanded in number before or after insertion of the genes. A method for
20 transforming blood cells is described in U.S. Pat. No. 5,286,497. Thus, the procedure is performed in such a manner that upon injection into the patient, the transformed blood cells will produce an anti-cancer agent in the patient's body, preferably at the site of the tumor itself.

The gene carried by the transformed stem cells can be any gene
25 which directly or indirectly enhances the therapeutic effects of the resultant mature blood cells. The gene carried by the stem cells can be any gene which allows the blood cells to exert a therapeutic effect that it would not ordinarily have, such as a gene encoding a clotting factor useful in the treatment of hemophilia. Examples of other suitable genes include those that encode
30 cytokines such as TNF, interleukins (interleukins 1-12), interferons (α , β , γ -

interferons), T-cell receptor proteins and Fc receptors for antigen-binding domains of antibodies, such as immunoglobulins.

Additional examples of suitable genes include genes that modify blood cells to “target” to a site in the body to which the blood cells would not ordinarily “target,” thereby making possible the use of the blood cell's therapeutic properties at that site. In this fashion, blood cells can be modified, for example, by introducing a Fab portion of a monoclonal antibody into the stem cells, thereby enabling the mature blood cells to recognize a chosen antigen. Other genes useful in cancer therapy can be used to encode chemotactic factors which cause an inflammatory response at a specific site, thereby having a therapeutic effect. Other examples of suitable genes include genes encoding soluble CD4 which is used in the treatment of AIDS and genes encoding preselected polypeptides or protein that can act to correct or ameliorate genetic disorders which result in insufficient or defective enzymes. Such genes include the α -antitrypsin gene, which is useful in the treatment of emphysema caused by α -antitrypsin deficiency, a tyrosine hydroxylase gene (Parkinson's disease), a glucocerebrosidase gene (Gaucher's disease), an “ α -galactosidase gene (Fabray's disease) an arylsulfatase A gene (metachromatic leukodystrophies) or genes encoding other polypeptides or proteins.

The gene therapy of the present invention is also useful in the treatment of a variety of diseases including but not limited to adenosine deaminase deficiency, sickle cell anemia, thalassemia, hemophilia, diabetes, “-antitrypsin deficiency, brain disorders such as Alzheimer's disease, and other illnesses such as growth disorders and heart diseases, for example, those caused by alterations in the way cholesterol is metabolized and defects of the immune system.

One of skill in the art would recognize that the conditions discussed herein above can have multiple causes and can overlap in naming and categorization.

Salts

In cases where complexes are sufficiently basic or acidic to form stable nontoxic acid or base salts, administration as salts may be appropriate. Examples of pharmaceutically acceptable salts are organic acid addition salts
 5 formed with acids which form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorbate, α -ketoglutarate, and α -glycerophosphate. Suitable inorganic salts may also be formed, including hydrochloride, sulfate, nitrate, bicarbonate, and carbonate salts.

10 Pharmaceutically acceptable salts may be obtained using standard procedures well known in the art, for example by reacting a sufficiently basic compound such as an amine with a suitable acid affording a physiologically acceptable anion. Alkali metal (for example, sodium, potassium or lithium) or
 15 alkaline earth metal (for example calcium) salts of carboxylic acids can also be made.

The amount of the complex or salt required for use in diagnosis or treatment will vary not only with the particular complex selected but also with the route of administration, the nature of the condition being treated and the age
 20 and condition of the patient, and will be ultimately at the discretion of the attendant physician or clinician. Typical dose ranges for imaging are 3-45 mCi per 70 kg body weight, preferably 5-35 mCi per 70 kg, more preferably 10-30 mCi per 70 kg. See Fritzberg et al. (U.S. Pat. No. 5,089,249). Typical dose ranges for therapy are 10-450 mCi per 70 kg body weight, preferably 20-300
 25 mCi per 70 kg, more preferably 40-200 mCi per 70 kg. See Fritzberg et al. (U.S. Pat. No 5,089,249).

The ability of a compound of the invention to suppress bone marrow growth can be determined using pharmacological models which are well known to the art. Examples of useful assays are found in Cleynhens, B. et
 30 al. (Technetium, Rhenium, and Other Metals in Chemistry and Nuclear Medicine (1999) p. 605), Larsen, R.H. et al. (Journal of Labelled Compounds and

Radiopharmaceuticals **XLI**, 823 (1998)), Fritzberg et al. (U.S. Pat. No. 5,089,249), and Simon et al. (U.S. Pat. No. 5,064,633).

The invention will now be illustrated by the following non-limiting Examples wherein unless otherwise noted: reagents were obtained from Aldrich Chemical Co. (Milwaukee, WI); anhydrous solvents were obtained from Acros Organics (Fisher Scientific, Pittsburg, PA); other solvents were reagent or HPLC grade and obtained from Fisher Scientific; air sensitive reactions were carried out under nitrogen or argon; proton NMR spectra were obtained on a Varian Gemini-200 spectrometer; chemical shifts are expressed in parts per million (δ) using residual solvent protons as internal standard; mass spectra were obtained using a ThermoQuest/Finnigan LCQ Detector (San Jose, CA) with an atmospheric pressure ionization (API) source and an electrospray ionization (ESI) probe; flash chromatography was carried out using Merck Silica Gel 60 (230-400 mesh); TLC was performed on pre-coated glass plates (Baker, Si250F with 254nm fluorescent indicator), and spots were detected using UV light, iodine, ninhydrin spray (0.2% in EtOH) or an aqueous solution containing phosphomolybdic acid, $\text{Ce}(\text{SO}_4)_2$ and H_2SO_4 ; reverse phase flash chromatography was carried out using Bakerbond octadecyl (C-18) 40 μm Prep LC packing; reversed phase TLC was performed on pre-coated glass plates (EM Science RP-18 F_{254S}); analytical HPLC was carried out using a Waters 600 multi-solvent delivery system and a Waters 490 programmable multiwavelength detector with a 5 μm C-18 column from Beckman Ultrasphere (Fullerton, CA); and preparative HPLC was performed on a Waters 3000 system with a Model 481 spectrophotometer (254 nm) using C-18 reversed phase columns from Microsorb (Walnut Creek, CA). Compounds **1**, **17** and **20** (Figures 4, 7, and 9) were synthesized by known literature procedures. (Yau, E.K.; Theodore, L.J.; Gustavson, L.M. *US Patent* 5,847,121 (Dec. 8, 1998); Kieczkowski, G.R.; Jobson, R.B.; Melillo, D.G.; Reinhold, D.F.; Grenda, V.J.; Shinkai, I. *J. Org. Chem.* **1995**, 60, 8310-8312; and Nanjappan, P.; Raju, N.; Ramalingam, K.; Nowotnik, D.P. *Tetrahedron* **1994**, 50, 8617-8632.)

Example 1. (6-{4-[2-(Bis-phosphonomethyl-amino)-acetylamino]-benzyl}-4,7,10-tris-carboxymethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-acetic acid (**8**, Figure 4).

5 To a solution of protected DOTA chelate **7** (49.2 mg; 45.1 μ mol) in CH_2Cl_2 (3 mL) was added TMSBr (600 μ L). The solution was stirred overnight, then H_2O (1 mL) was added and stirring was continued for 30 minutes. Concentration yielded 66.1 mg of crude material which was purified by preparative HPLC on a C-18 reversed phase column using 100% H_2O
 10 (containing 0.1% TFA) as the eluting solvent: Yield = 15.6 mg (29%); ^1H NMR (D_2O) δ 7.41 (d, 2H), 7.25 (d, 2H), 4.49 (s, 2H), 4.37-2.48 (complex multiplet, 25H), 3.68 (d, 4H); ESI-MS 755.2 (M+H), 777.2 (M+Na).

The intermediate protected DOTA chelate **7** was prepared as
 15 follows.

a. Tetra t-butyl nitrobenzyl DOTA **2** (Figure 4). To a rapidly stirred solution of (S)-2-(p-Nitrobenzyl)-1,4,7,10-tetraazacyclododecane trihydrochloride salt **1** (350 mg; 0.84 mmol) and K_2CO_3 (1.16 g; 8.4 mmol) in
 20 anhydrous DMF (7 mL) was added t-butyl bromoacetate (0.62 mL; 4.2 mmol). The mixture was then heated to 50-55°C and the progress of the reaction was monitored by HPLC. After 3 hours the mixture was cooled to room temperature then stored at 4°C overnight. The precipitated solid was collected and rinsed with additional DMF (5 mL). This yielded 786 mg of crude material which was
 25 purified by silica gel chromatography, eluting with 0-10% MeOH/ CH_2Cl_2 , to yield 528 mg (82%) of **2**. R_f = 0.30 (10% MeOH/ CH_2Cl_2); ^1H NMR (CDCl_3) δ 8.17 (dd, 2H), 7.60 (d, 1H), 7.40 (d, 1H), 3.78-1.62 (complex multiplet, 25H), 1.49-1.37 (4s, 36H); ESI-MS 786.4 [M+Na].

30 b. Tetra t-butyl aminobenzyl DOTA **3** (Figure 4). To a solution of nitrobenzyl compound **2** (528.7 mg; 0.69 mmol) in MeOH (50 mL) in a Parr

shaker bottle was added 10% Pd/C (100 mg). The solution was then placed on the Parr apparatus under a hydrogen atmosphere of 50 psi. After 3 hours the solution was filtered and concentrated to yield 472 mg of crude material.

Purification by silica gel chromatography, eluting with 0-10% MeOH/CH₂Cl₂ yielded 370 mg (73%) of **3**. R_f = 0.27 (10% MeOH/CH₂Cl₂); ¹H NMR (CDCl₃) δ 6.75 (dd, 2H), 6.58 (dd, 2H), 3.43-1.45 (complex multiplet, 25H), 1.38-1.29 (4s, 36H).

c. Protected DOTA chelate 7 (Figure 4). To a solution of aminobenzyl DOTA **3** (80.7 mg; 110 μmol) and amino acid hydrochloride **6** (50 mg; 121 μmol) in DMF (3 mL) was added benzotriazol-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP; Chem-Impex International; Wood Dale, IL; 53.5 mg; 121 μmol) and DIEA (154 μL; 883 μmol). The mixture was stirred under argon at room temperature overnight then concentrated. The crude residue was dissolved in CH₂Cl₂ (20 mL) then washed with H₂O (2x 10mL) and brine (10 mL) then dried (MgSO₄). Concentration gave 141 mg of crude material which was purified by silica gel chromatography, eluting with 0-8% MeOH/CH₂Cl₂, to yield 98.4 mg (82%) of protected DOTA chelate **7**: R_f = 0.45 (10% MeOH/CH₂Cl₂); ¹H NMR (CDCl₃) δ 7.60 (dd, 2H), 7.07 (dd, 2H), 4.15 (pentet, 8H), 3.60 (s, 2H), 3.21 (d, 4H), 3.50-1.70 (complex multiplet, 25H), 1.49 (s, 9H), 1.47 (s, 9H), 1.44 (s, 9H), 1.41 (s, 9H), 1.33 (t, 12H).

The intermediate amino acid hydrochloride **6** was prepared as follows.

d. Methyl ester 5 (Figure 4). (see U.S. Patents 5,714,604, and 4,976,950). To a solution of paraformaldehyde (1.32 g; 44 mmol) and glycine methyl ester hydrochloride **4** (2.51 g; 20 mmol) in anhydrous THF (30 mL) was added triethylphosphite (7.54 mL; 44 mmol). The mixture was heated to reflux for 16 hours then cooled to room temperature and concentrated *in vacuo*. The

material was dissolved in CH_2Cl_2 (100 mL) and washed sequentially with saturated aqueous NaHCO_3 solution (2x 50 mL), H_2O (50 mL) and saturated aqueous NaCl solution (50 mL). Drying (MgSO_4) and concentration yielded 7.208 g (93%) of methyl ester **4** which was of sufficient purity to be used in subsequent steps: ^1H NMR (CDCl_3) δ 4.12 (m, 8H), 3.82 and 3.73 (2s, 2H), 3.69 and 3.65 (2s, 3H), 3.25 (t, 4H), 1.31 (t, 12H); ESI-MS 389.9 $[\text{M}+\text{H}]$ and 412.1 $[\text{M}+\text{Na}]$.

e. Amino acid hydrochloride **6** (Figure 4). To a solution of methyl ester **5** (7.208 g; 18.5 mmol) in MeOH (30 mL) was added H_2O (15 mL) followed by the dropwise addition of 1N-NaOH (22 mL). The mixture was stirred at rt overnight then concentrated. The crude material was dissolved in H_2O (200 mL) and the aqueous solution washed with CH_2Cl_2 (2 x 100 mL) then acidified to pH~2 by the addition of 1N-HCl (22 mL). The aqueous solution was saturated with NaCl then extracted with 3:1 CH_2Cl_2 : $i\text{-PrOH}$ (3x 150 mL). Drying (MgSO_4) and concentration *in vacuo* yielded 3.519 g (46%) of acid **6** which was of sufficient purity to be used in subsequent steps: ^1H NMR (CDCl_3) δ 4.10 (pentet, 8H), 3.70 (s, 2H), 3.28 (d, 4H), 1.27 (t, 12H).

Example 2. (6-{4-[4-(Bis-phosphonomethyl-carbamoyl)-butyrylamino]-benzyl}-4,7,10-tris-carboxymethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-acetic acid (**11**, Figure 5).

A solution of tetra t-butyl ester **10** (23.2 mg; 16.9 μmol) in TFA (5 mL) was stirred at room temperature overnight. Concentration followed by dissolution in H_2O (12 mL) and lyophilization yielded 16.5 mg (85%) of **11** as a feathery white solid which was shown by HPLC to be of >98% purity: ^1H NMR (D_2O) δ 7.34 (d, 2H); 7.21 (dd, 2H), 4.43-2.69 (complex multiplet, 27H), 2.58 (t, 2H), 2.40 (t, 2H), 1.92 (m, 2H); ESI-MS 811.2 ($\text{M}+\text{H}$), 833.2 ($\text{M}+\text{Na}$).

The intermediate tetra t-butyl ester **10** was prepared as follows.

- a. DOTA linker acid 9 (Figure 5). To a solution of DOTA aniline **3** (110.6 mg; 151 μ mol) and glutaric anhydride (34.3 mg; 301 μ mol) in anhydrous DMF (8 mL) was added DIEA (0.26 mL; 1.51 mmol). The solution was stirred overnight then concentrated *in vacuo* to yield 146 mg of crude material.
- 5 Purification by preparative HPLC on a C-18 reversed phase column, eluting with H₂O/MeCN containing 0.1% TFA (using a gradient of 40-64% MeCN over 30 min) yielded 121 mg (67%) of **9** as its tri TFA salt: ¹H NMR (CD₃OD) δ 7.55 (m, 2H), 7.30 (m, 2H), 4.53-2.52 (complex multiplet, 25H), 2.42 (2t, 4H), 1.97 (pentet, 2H), 1.70-1.40 (4s, 36H); ESI-MS 848.4 (M+H), 870.4 (M+Na).
- 10
- b. Protected DOTA chelate 10 (Figure 5). To a solution of acid **9** (45.2 mg; 38 μ mol) in DMF (3 mL) was added BOP (18.5 mg; 41.8 μ mol) followed by diisopropylethylamine (99 μ L; 570 μ mol). The solution was stirred, under argon, at room temperature for 30 min then iminobis(methylphosphonic acid) (8.6 mg; 41.8 μ mol) was added. Progress of the reaction was monitored by
- 15 HPLC. After 7 hours the solution was concentrated to give 107.4 mg of crude material, which was purified by preparative HPLC on a C-18 reversed phase column eluting with H₂O/MeCN containing 0.1% TFA (using a gradient of 40-64% H₂O over 30 min). This yielded 23.2 mg (44%) of **10** as its tri TFA salt:
- 20 ESI-MS 518.3 ([M+2H]/2), 1035.3 (M+H), 1057.3 (M+Na).

Example 3. {3-[4-(3,3-Bis-phosphono-propionylamino)-benzyl]-4,7,10-tris-carboxymethyl-1,4,7,10-tetraaza-cyclododec-1-yl}-acetic acid (**16**, Figure 6).

- 25 To a solution of compound **15** (132 mg; 124 μ mol, Figure 6) in CH₂Cl₂ (4 mL) was added trimethylsilyl bromide (1.25 mL). The mixture was stirred, under nitrogen, overnight. Water (1 mL) was added and stirring continued for 30 minutes. Concentration yielded 229 mg of crude material which was purified by preparative HPLC on a C-18 reversed phase column eluting with
- 30 H₂O/MeCN containing 0.1% TFA (using a gradient of 0%-8% MeCN over 30 min). This yielded 49.7 mg (38%) of **16** as its tri trifluoroacetic acid salt: ¹H

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NMR (D₂O) δ 7.31 (d, 2H), 7.16 (d, 2H), 4.39-2.40 (complex multiplet, 28H);
ESI-MS 726.2 (M+H).

The intermediate compound **15** was prepared as follows.

5

a. t-Butyl ester **13** (Figure 6, see Ebetino, F.H., et al., *Heterocycles* **1990**, *30*, 855-862). To a slurry of KH (160 mg; 4.0 mmol) in mineral oil, under argon, was added anhydrous toluene (4 mL). The mixture was stirred for approximately 30 seconds then the solvents removed via syringe. This process
10 was repeated. Additional toluene (4 mL) was added and the solution cooled in ice. A solution of tetraethyl methylenediphosphonate (0.75 mL; 3.0 mmol) in anhydrous toluene (8 mL) was added dropwise over 5 minutes. The ice bath was removed and the solution allowed to warm to rt. After 2 hours t-butyl
15 bromoacetate (0.44 mL; 3.0 mL) was added dropwise. The solution was then heated to 80-85°C for 2 hours then cooled to room temperature and quenched via the dropwise addition of MeOH (1 mL). Concentration followed by dissolution in CH₂Cl₂ (20 mL) and filtration (to remove insoluble KBr) then further concentration yielded 1.109 g (92%) of t-butyl ester **13**. This material was of sufficient purity to be used in subsequent experiments: ¹H NMR (CDCl₃) δ 4.18
20 (pentet, 8H), 3.21 (br s, 1H), 2.75 (dd, 2H), 1.45 (s, 9H), 1.31 (t, 12H); ESI-MS 425.0 (M+Na), 440.9 (M+K).

b. Acid **14** (Figure 6). A solution of t-butyl ester **13** (555 mg; 1.4 mmol) in TFA (10 mL) was stirred at room temperature under nitrogen for 6
25 hours then concentrated. A quantitative yield was assumed and the material used immediately in the subsequent step: ¹H NMR (CDCl₃) δ 4.19 (pentet, 8H), 3.15 (m 1H), 2.88 (dt, 2H), 1.37 (t, 12H).

c. Protected DOTA chelate **15** (Figure 6). To a solution of acid **14**
30 (83.1 mg; 240 μ mol) and amine **3** (147 mg; 200 μ mol) in DMF (3 mL) was added BOP (106 mg; 240 μ mol) followed by diisopropylethyl amine (350 μ L);

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2.0 mmol). The solution was stirred, under nitrogen, at rt overnight then concentrated. The crude material was purified by flash chromatography (SiO₂; 0-7.5% MeOH/CH₂Cl₂) to give 132 mg (62%) of **15**: R_f = 0.46 (10% MeOH/CH₂Cl₂); ¹H NMR (CDCl₃) δ 7.53 (dd, 2H), 7.03 (dd, 2H), 4.15 (m, 8H), 3.50-1.70 (complex multiplet, 28H) 1.45-1.35 (4s, 36H), 1.32 (m, 12H); ESI-MS 1084.4 (M+Na).

Example 4. (4,7,10-Tris-carboxymethyl-3-{4-[4-(3-hydroxy-3,3-bis-phosphono-propylcarbamoyl)-butyrylamino]-benzyl}-1,4,7,10-tetraaza-cyclododec-1-yl)-acetic acid (**19**, Figure 7).

A solution of compound **18** (32.4 mg; 22.7 μmol) in TFA (5 mL) was stirred under nitrogen at room temperature overnight. Concentration yielded 36.4 mg of crude material. Purification by preparative HPLC on a C-18 reversed phase column eluting with H₂O/MeCN containing 0.1% TFA (using a gradient of 0-16% MeCN over 30 min) yielded 18.6 mg (69%) of **19** as its tri TFA salt: ¹H NMR (D₂O) δ 7.43 (m, 2H), 7.28 (m, 2H), 4.27-2.35 (complex multiplet, 36H); ESI-MS 533.8 ([M+H+Na]/2), 1042.3 (M+H), 1066.2 (M+Na).

The intermediate compound **18** was prepared as follows.

a. Compound **18** (Figure 7). To a solution of acid **9** (59.9 mg; 50.3 μmol) in DMF (3 mL) was added BOP (26.7 mg; 60.4 μmol) followed by DIEA (87.7 μL; 503 μmol). The mixture was stirred for 10 minutes then a solution of the amino diphosphonate **17** (64.7 mg; 251.6 μmol) in H₂O (2 mL) was added followed by additional DIEA (35 μL). Progress of the reaction was monitored by HPLC. After 16 hours the solution was filtered and the filter cake rinsed with DMF (5 mL). Concentration of the mother liquor yielded 143 mg of crude material which was purified by preparative HPLC on a C-18 reversed phase column eluting with H₂O/MeCN containing 0.1% TFA (using a gradient of 40-64% MeCN over 30 min) to provide 32.4 mg (45%) of compound **18** as its tri

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TFA salt: ^1H NMR (CD_3OD) δ 7.56 (br s, 2H), 7.30 (br s, 2H), 4.55-1.73 (complex multiplet, 35H), 1.65-1.38 (4s, 36H); ESI-MS 1065.3 (M+H), 1087.3 (M+Na).

- 5 **Example 5.** {3-[4-(3-[2-(Bis-phosphonomethyl-amino)-acetylamino]-2-[[2-(bis-phosphonomethyl-amino)-acetylamino]-methyl]-propionylamino)-benzyl]-4,7,10-tris-carboxymethyl-1,4,7,10-tetraaza-cyclododec-1-yl}-acetic acid (**24**, Figure 8).

- 10 To a solution of compound **23** (25.9 mg; 12.2 μmol) in CH_2Cl_2 (3 mL) under nitrogen was added TMSBr (0.5 mL). The solution was stirred overnight then cooled in an ice bath. H_2O (3 mL) was added and stirring continued for 30 minutes. Concentration yielded 23.9 mg of crude product. Purification by preparative HPLC on a C-18 reversed phase column eluting with
- 15 $\text{H}_2\text{O}/\text{MeCN}$ containing 0.1% TFA (using a gradient of 0-8% MeCN over 30 min) yielded 8.7 mg (43%) of **24** as its penta TFA salt: ^1H NMR (D_2O) δ 7.37 (d, 2H), 7.17 (d, 2H), 4.47-2.48 (complex multiplet, 27H), 2.40 (t, 2H), 2.28 (t, 2H), 2.15 (m, 2H), 1.93 (m, 2H); ESI-MS 841.4 (M+H), 879.2 (M+K).

- 20 The intermediate compound **23** was prepared as follows.

- a. Tetraphosphonate methyl ester **21** (Figure 8). To a solution of bisamine **20** (51.3 mg; 0.25 mmol) and acid **6** (218 mg; 0.53 mmol) in anhydrous DMF (4 mL) was added BOP (234 mg; 0.53 mmol) followed by DIEA (0.65 mL;
- 25 3.75 mmol). The mixture was stirred, under nitrogen, at room temperature overnight, then concentrated to give 635 mg of crude material. Purification by flash chromatography (SiO_2 ; 0-10% MeOH/ CH_2Cl_2 containing 0.1% Et_3N) yielded 428 mg (>100%) of **21** which still contained some HMPA impurity. This material was used in subsequent steps without further purification: R_f = 0.27
- 30 (10% MeOH/ CH_2Cl_2 + 0.1% Et_3N); ^1H NMR (CDCl_3) δ 7.71 (t, 2NH), 4.12

(pentet, 16H), 3.78-3.40 (m, 4H), 3.69 (s, 3H), 3.50 (s, 2H), 3.22 (m, 8H), 2.92 (m, 1H), 1.32 (t, 24H); ESI-MS 847.0 (M+H), 869.2 (M+Na).

- b. Tetraphosphonate carboxylic acid **22** (Figure 8). To a solution of methyl ester **21** (153.9 mg; 167 μ mol) in MeOH (3 mL) and water (3 mL) was added 1N-NaOH (1 mL). The mixture was stirred overnight then concentrated and the crude residue dissolved in H₂O (5 mL). The aqueous solution was acidified to pH~2 with 1N-HCl (1 mL) then concentrated. MeCN (5 mL) was added to the residue and the insoluble NaCl removed by filtration.
- Concentration of the mother liquor yielded 101 mg (67% for 2 steps) of acid **22** which was of sufficient purity to be used in subsequent experiments: ¹H NMR (CDCl₃) δ 7.78 (t, 2NH), 4.11 (multiplet, 16H), 3.80-3.35 (m, 8H), 3.48 (s, 4H), 3.17 (d, 8H), 2.83 (m, 1H), 1.31 (t, 24H); ESI-MS 833.0 (M+H), 855.2 (M+Na).
- c. Compound **23** (Figure 8). To a solution of amine **3** (82 mg; 112 μ mol) and acid **22** (101.3 mg; 112 μ mol) in anhydrous DMF (3 mL) was added BOP (49.5 mg; 112 μ mol) followed by DIEA (293 μ L; 1.68 mmol). The mixture was stirred, under nitrogen, at room temperature overnight then concentrated to give 323 mg of crude material. Purification by flash chromatography (SiO₂; 0-10% MeOH/CH₂Cl₂ containing 0.1% Et₃N) yielded 64 mg of **23** which was further purified by preparative HPLC on a C-18 reversed phase column, eluting with H₂O/MeCN containing 0.1% TFA (using a gradient of 40-64% MeCN over 30 min) to yield 25.9 mg (11%) of **23** as its penta TFA salt: R_f = 0.11 (10% MeOH/CH₂Cl₂ + 0.2% Et₃N); ¹H NMR (CDCl₃) δ 7.47 (d, 2H), 7.13 (d, 2H), 4.11 (pentet, 16H), 4.00-2.28 (complex multiplet, 38H), 3.48 (s, 4H), 1.59-1.42 (4s, 36H), 1.35 (t, 24H); ESI-MS 774.9 ([M+2H]/2), 1548.5 (M+H), 1570.6 (M+Na).

Example 6. [3-(4-{3-(3,3-Bis-phosphono-propionylamino)-2-[(3,3-bis-phosphono-propionylamino)-methyl]-propionylamino}-benzyl)-4,7,10-tris-carboxymethyl-1,4,7,10tetraaza-cyclododec-1-yl]-acetic acid (**28**, Figure 9).

5 To a solution of compound **27** (64 mg; 43.0 μ mol) in CH_2Cl_2 (4 mL) under nitrogen was added TMSBr (0.5 mL). The solution was stirred overnight then cooled in an ice bath. H_2O (1 mL) was added and stirring continued for 30 minutes. Concentration yielded 78.6 mg of crude product. Purification by preparative HPLC on a C-18 reversed phase column eluting with
10 $\text{H}_2\text{O}/\text{MeCN}$ containing 0.1% TFA (using a gradient of 0-20% MeCN over 30 min) yielded 26.5 mg (45%) of compound **28** as its tri TFA salt: ^1H NMR (D_2O) δ 7.43 (m, 2H), 7.28 (m, 2H), 4.27-2.35 (complex multiplet, 36H); ESI-MS: 533.8 ($[\text{M}+\text{H}+\text{Na}]/2$), 1042.3 (M+H), 1066.2 (M+Na).

15 The intermediate compound **27** was prepared as follows.

a. Tetraphosphonate methyl ester **25** (Figure 9). To a solution of bisamine **20** (51.3 mg; 250 μ mol) and acid **14** (190.4 mg; 550 μ mol) in anhydrous DMF (4 mL) was added BOP (243 mg; 550 μ mol) followed by DIEA
20 (871 mL; 5 mmol). The mixture was stirred, under nitrogen, at room temperature overnight, then concentrated. The residue was dissolved in CH_2Cl_2 (20 mL) and washed with 1N-HCl (2x 10 mL), H_2O (10 mL) and saturated aqueous NaHCO_3 solution (10 mL). Drying (MgSO_4) and concentration yielded 224 mg of crude material. Purification by flash chromatography (SiO_2 ; 0-10% MeOH/ CH_2Cl_2)
25 yielded 96.4 mg (49%) of **25**: R_f = 0.32 (10% MeOH/ CH_2Cl_2); ^1H NMR (CDCl_3) δ 7.53 (t, 2NH), 4.09 (m, 16H), 3.68 (m, 2H), 3.62 (s, 3H), 3.45-3.09 (m, 4H), 2.98 (m, 1H), 2.65 (dt, 4H), 1.26 (t, 24H); ESI-MS 789.0 (M+H), 811.2 (M+Na), 827.1 (M+K).

30 b. Tetraphosphonate carboxylic acid **26** (Figure 9). To a solution of methyl ester **25** (95 mg; 120 μ mol) in MeOH (2 mL) and H_2O (1 mL) was added

1N-NaOH (150 μ L). The solution was stirred at room temperature overnight then concentrated. The residue was dissolved in H₂O (5 mL) then acidified to pH~2 with 1N-HCl (150 μ L). The aqueous solution was extracted with 3:1 CH₂Cl₂:ⁱPrOH (3x 5 mL) and the combined organics dried (Na₂SO₄) and concentrated to
5 give 88.1 mg (95%) of **26**, which was of sufficient purity to be used in subsequent steps: ¹H NMR (CD₃OD) δ 8.19 (t, 2NH), 4.18 (pentet, 16H), 3.63-3.11 (m, 6H), 2.81 (m, 1H), 2.72 (dt, 4H), 1.33 (t, 24H); ESI-MS 775.0 (M+H), 797.2 (M+Na).

10 c. Compound 27 (Figure 9). To a solution of amine **3** (83 mg; 114 μ mol) and acid **26** (88 mg; 114 μ mol) in anhydrous DMF (3 mL) was added BOP (50 mg; 114 μ mol) followed by DIEA (200 μ L; 0.57 mmol). The mixture was stirred, under nitrogen, at room temperature overnight, then concentrated to give 259 mg of crude material. Purification by flash chromatography (SiO₂;
15 0-10% MeOH/CH₂Cl₂) yielded 128.3 mg (79%) of **27**: TLC R_f = 0.38 (10% MeOH/CH₂Cl₂); ¹H NMR (CDCl₃) δ 7.54 (d, 2H), 7.15 (dd, 2H), 4.14 (m, 16H), 3.65-1.70 (complex multiplet, 36H), 1.50-1.38 (4s, 36H), 1.33 (t, 24H); ESI-MS 746.0 ([M+2H]/2), 756.9 ([M+H+Na]/2), 768.0 ([M+2Na]/2), 1490.4 (M+H), 1512.5 (M+Na).

20 All publications, patents, and patent documents are incorporated by reference herein, as though individually incorporated by reference. The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and
25 scope of the invention.